

# Fungus Invasion into Human Hair Tissue in Black Dot Ringworm: Light and Electron Microscopic Study

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In order to investigate the morphology of fungi invading into the human hair tissue, three cases of black dot ringworm caused by *Trichophyton violaceum* and *Trichophyton glabrum* were studied by light and electron microscopy. Fungal elements were mainly present in the hair cortex and showed a constant morphologic change during the differentiation of hair layers. The fungal elements, located deep in the keratogenous zone of the cortex, showed less electron dense non-septate hyphae. Distally, the hyphae showed septation and contained several scattered dense bodies in the cytoplasm. At the level where the Huxley's layer was keratinized, the fun-

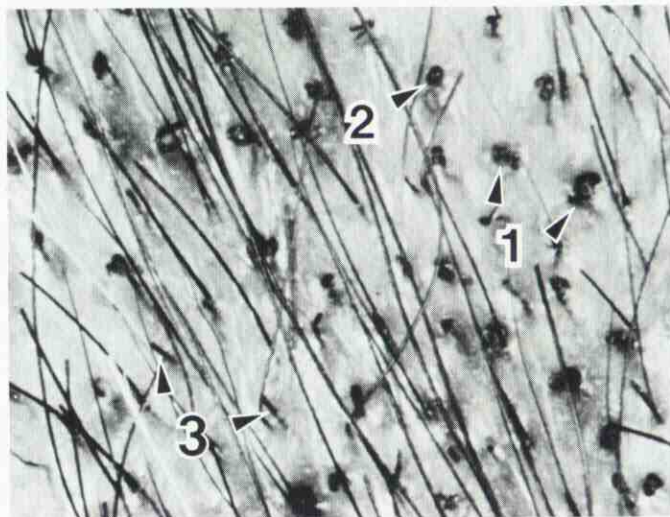
gal elements were transformed into arthrospores, which occupied the large volume of the cortex; each spore was surrounded by a fiber- and melanosome-free, electron lucent halo. Fungal elements occasionally invaded the keratinized hair cuticle and keratinized inner root sheath in a few hair follicles. Fungi do not invade the hair germinative cells. There seems to be a distinct relationship between the morphology of the invading fungi and the cortical cell differentiation in black dot ringworm; a balance between the fungus proliferation and the cortical cell development may be present. *J Invest Dermatol* 90:729-733, 1988

In tinea capitis, affected hairs have been studied by light microscopy (LM) [1,2], scanning electron microscopy [3-6], and transmission electron microscopy (TEM) [7-9]. However, no complete morphologic description of the parasitic form of dermatophytes in the hair tissue has been made, and the pathologic changes of the abnormal hair structure have not been sufficiently known. The in vitro invasion into human hair by keratinophilic fungi [10-13] does not seem to reflect exactly the in vivo pathologic change. To investigate the morphology of fungi invading into human hair tissue and the pathogenesis in tinea capitis, scalp lesions of three cases of black dot ringworm, which is known to be one of tinea capitis due to intrapilar invasion of dermatophytes, were examined by LM and TEM in the present study.

## MATERIALS AND METHODS

The patients were 72-, 75-, and 73-year-old Japanese females. In the scalp lesions, many hairs were broken at the level of the skin surface and mingled with hairs growing normally. Although some of the broken hairs showed simply cut ends 1-3 mm above the skin surface, many showed coiling at the similar level or in the pilar pores; these coiled hair masses appear as black dots (Fig 1). Slight erythema was partly seen in the lesions; however, neither scaling nor pustule formation was seen. In all the cases, prior to biopsy, the diagnosis of tinea capitis was established by demonstration of intrapilar fungal elements by direct LM examination. Hair samples from the lesions yielded *Trichophyton violaceum* in the former two cases and *Trichophyton glabrum* in the last on Sabouraud's dextrose agar. Biopsied skin specimens were obtained from the scalp lesions of the three

cases, double-fixed in 5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) and 2% osmium tetroxide in the same buffer, dehydrated in a series of graded ethanol solutions and propylene oxide, and embedded in Epon 812. Longitudinal thick sections of 80 hair follicles were made with a Sorvall MT 5000 ultramicrotome and glass knives and stained with toluidine blue. Fifty anagen hair follicles were affected by the fungi; ultrathin sections of these affected hair follicles were cut with the same ultramicrotome and a diamond knife, double-stained with uranyl acetate and lead citrate [14], and observed in a JEM 100S TEM. Ultrathin cross-sections of several affected hair follicles at varied levels were similarly made and examined.



**Figure 1.** Clinical feature of scalp lesion of black dot ringworm. 1, coiled hair masses in the pilar pores; 2, broken hair showing coiling above the skin surface; 3, broken hairs showing a simply cut end above the skin surface.

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Abbreviations:

LM: light microscopy

TEM: transmission electron microscopy



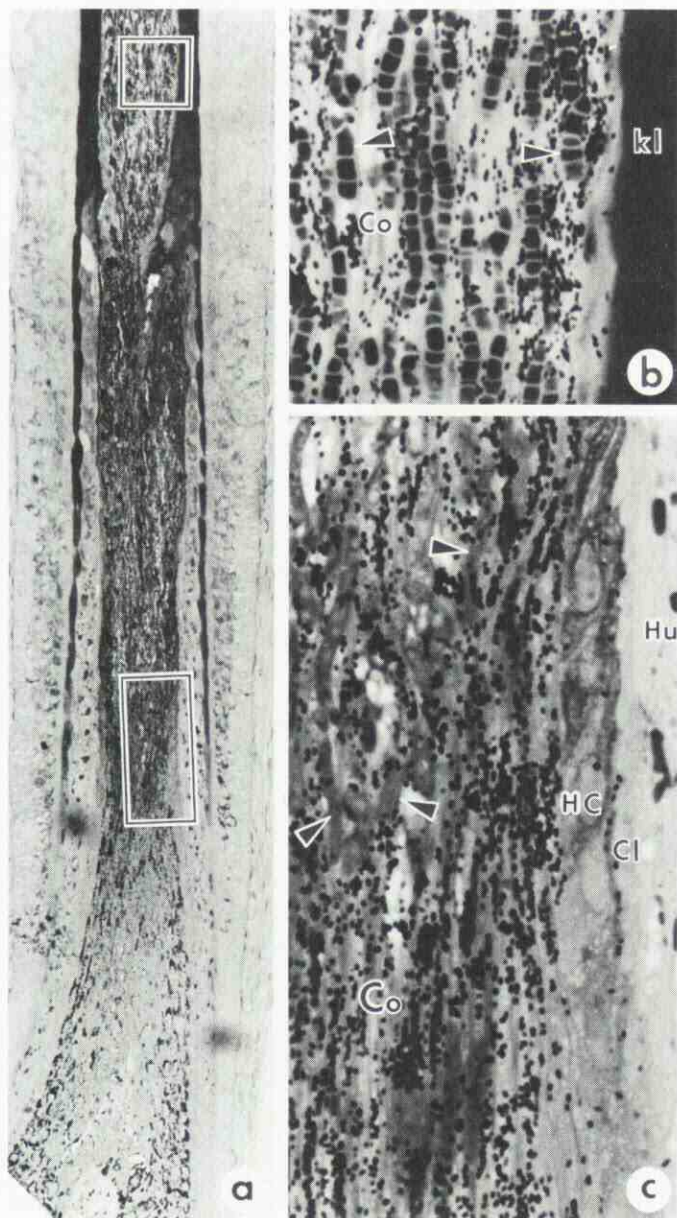
## RESULTS

All three cases revealed similar histopathological and ultrastructural findings.

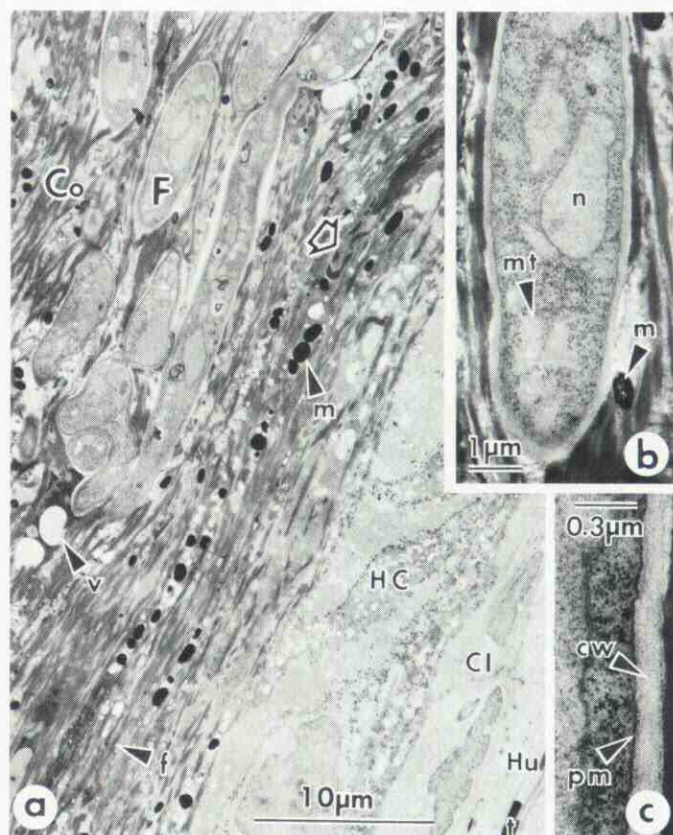
**LM Findings** Many of the affected hairs were broken around the level of the skin surface with or without coiling and some affected hairs as well as nonaffected hairs still preserved their straight hair shafts. A larger number of fungal elements was seen in the coiled affected hairs than in the straight ones. The fungal elements usually appeared to be present in the cortex (Fig 2a). The entire structures of the affected hair follicles were well preserved, except for some

affected hair follicles showing dilatation of the upper follicular epithelium due to coiling of the hair shaft. The fungal elements located in the proximal portion of the cortex were stained paler with toluidine blue (Fig 2c) than those distally located (Fig 2b). No inflammatory cell infiltrate was seen. Five telogen follicles were observed; only one telogen follicle was affected by the fungi.

**TEM Findings** The hair bulbs of all the hair follicles examined were intact. In 48 out of 50 affected hair follicles the fungal elements invaded and reached the keratogenous zone of the cortex, where the nuclei of the cortical cells were going to disappear; at this level, the fungal elements showed less electron dense nonseptate hyphae and measured about  $3\ \mu\text{m}$  in width. The fungal elements were not inserted between the cortical structures, but they replaced some parts of the structures, such as bundles of tonofilaments and melanosomes. Some vacuoles containing amorphous material with a low electron density were scattered in the cortex (Fig 3a). The hyphae contained some organelles such as mitochondria and ribosomes [15] and had a homogeneous cell wall of  $0.1\text{--}0.15\ \mu\text{m}$  thickness (Fig 3b,c). At the level where the hair cuticle was keratinized and the Huxley's layer was producing large trichohyaline granules, the hyphae showed a width of  $3\text{--}3.5\ \mu\text{m}$  and septation (Fig 4a-c) occasionally with septal pores (Fig 4c). They had a cell wall of approximately  $0.2\text{--}0.25\ \mu\text{m}$  thickness; in the cell wall, electron dense multilayered fibrillar structure was frequently recognized (Fig 4c). In addition to the organelles seen in the nonseptate hyphae, the

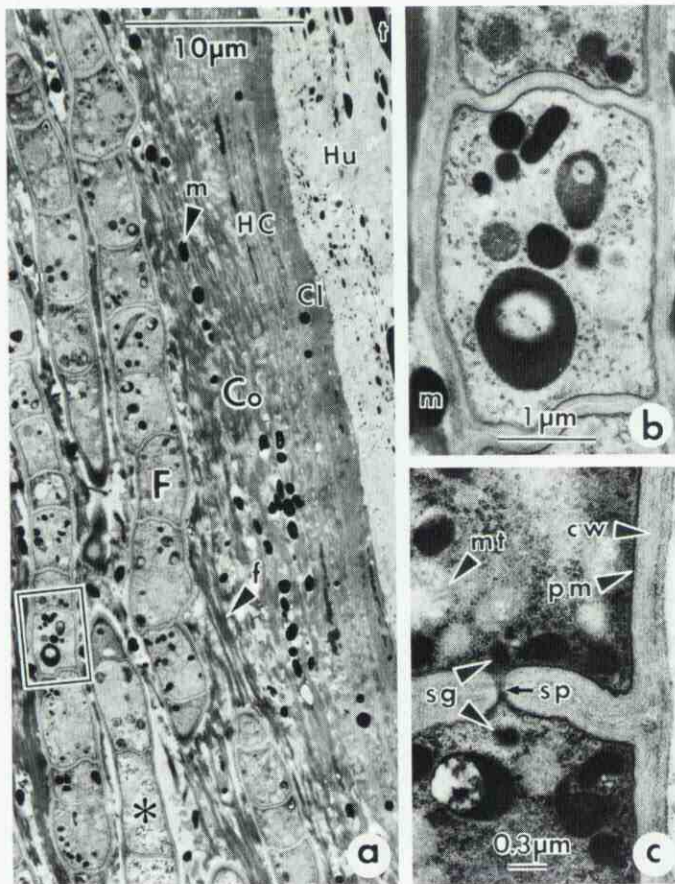


**Figure 2.** Light micrographs of a longitudinal section of the affected hair tissue stained with toluidine blue. *a*, hair bulb through keratogenous zone. Fungal elements are present in the hair cortex. All the hair layers look like they are developing normally and keratinizing. *b*, enlargement of the area indicated by the upper enclosure in *a*. Septate fungal elements (arrowheads), which show a dark stainability, are seen in the hair cortex (Co). *c*, enlargement of the area indicated by the lower enclosure in *a*. The fungal elements (arrowheads) showing a pale stainability are seen in the hair cortex (Co). CI, cuticle of inner root sheath; HC, hair cuticle; Hu, Huxley's layer; kI, keratinized inner root sheath. *a*,  $\times 120$ ; *b* and *c*,  $\times 800$

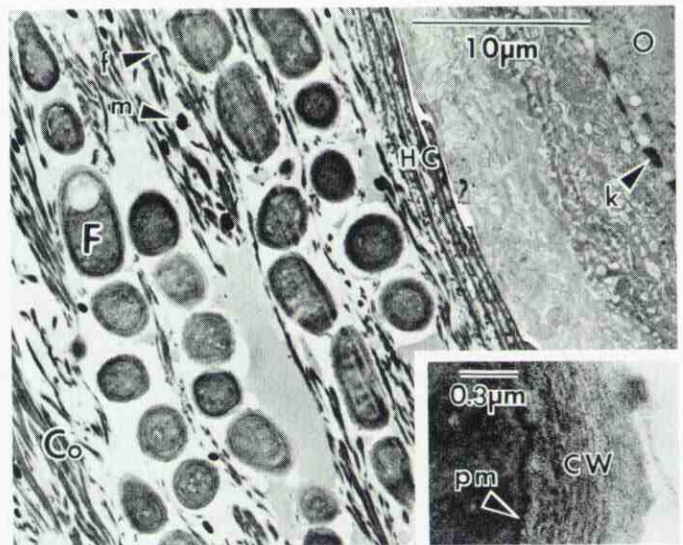


**Figure 3.** TEM findings of the lower keratogenous zone of the affected hair tissue. *a*, the area shown in Fig 2c. Nonseptate hyphae (F) with a low electron density are observed in the hair cortex (Co), replacing some parts of the cortical structure. *b*, enlargement of the fungal element indicated by F in Fig 3a. *c*, cell wall of the nonseptate hypha. Uranyl acetate-lead citrate stain. Arrow, disappearing nucleus of hair cortex; CI, cuticle of inner root sheath; cw, cell wall; f, tonofilaments; HC, hair cuticle; Hu, Huxley's layer; m, melanosome; mt, mitochondrion; n, nucleus; pm, plasma membrane; t, trichohyaline granule; v, vacuole in the hair cortex. *a*,  $\times 2,400$ ; *b*,  $\times 6,900$ ; *c*,  $\times 26,400$





**Figure 4.** TEM findings of the upper keratogenous zone of the affected hair tissue. *a*, longitudinal section of the hair tissue at the level where both the hair cuticle (HC) and cuticle of inner root sheath (CI) are keratinized. Septate hyphae (F) are observed in the hair cortex (Co). *b*, enlargement of the enclosed septate hypha. Dense bodies showing varied sizes and electron densities are irregularly scattered in the fungal cytoplasm. *c*, septal granules (sg) in the cytoplasm near the septal pore (sp) and multilayered, fibrillar structure in the cell wall (cw). Uranyl acetate-lead citrate stain. Asterisk, partially degenerated fungal cell; *f*, keratin filaments; *Hu*, Huxley's layer; *m*, melanosome; *mt*, mitochondrion; *pm*, plasma membrane; *t*, trichohyaline granule. *a*,  $\times 2,400$ ; *b*,  $\times 13,200$ ; *c*,  $\times 16,800$ .



**Figure 5.** TEM findings of a longitudinal section of the isthmus portion of the affected hair tissue, where the keratinized inner root sheath is degenerated. Fungal elements (F) in the hair cortex (Co) show the form of arthrospore. Around the fungal elements, halolike, fiber- and melanosome-free spaces with or without amorphous materials are observed. *Inset*, enlargement of the cell wall (cw) of arthrospore. Uranyl acetate-lead citrate stain. *f*, keratin filaments; *HC*, hair cuticle; *k*, keratohyaline granule; *m*, melanosome; *O*, outer root sheath; *pm*, plasma membrane.  $\times 2,400$ ; inset,  $\times 26,400$ .

In two affected hair follicles, the fungal elements invaded the cortex down to its developing zone just above the hair bulb; the developing cortical cells, having oval nuclei and a small number of tonofilaments in the cytoplasm, showed cell edema and an abrupt cell condensation or keratinization (Fig 6*a, b*). The fungal elements (nonseptate hyphae with a low electron density) were present in the condensed cortex (Fig 6*c*) but not in the edematous cortex. In the condensed cortical cells, pyknotic nuclei and incompletely aggregated tonofilaments were observed with degenerated cell organelles (Fig 6*b*). An early keratinization of Huxley's cells was also seen (Fig 6*a*).

Although fungal elements were usually seen within the cortex, they occasionally invaded the keratinized hair cuticle and keratinized inner root sheath; however, the outer root sheath was never invaded until it underwent keratinization at the isthmus and infundibular levels. In a rare instance, the innermost cell layer [19] of the outer root sheath was keratinized early near a fungal element invading the keratinized Henle's layer. In some affected hair follicles, in which fungi massively proliferated at the upper level, most of the cortex and cuticle structures were completely replaced by the fungal elements with surrounding halo spaces, leaving a small number of melanosomes.

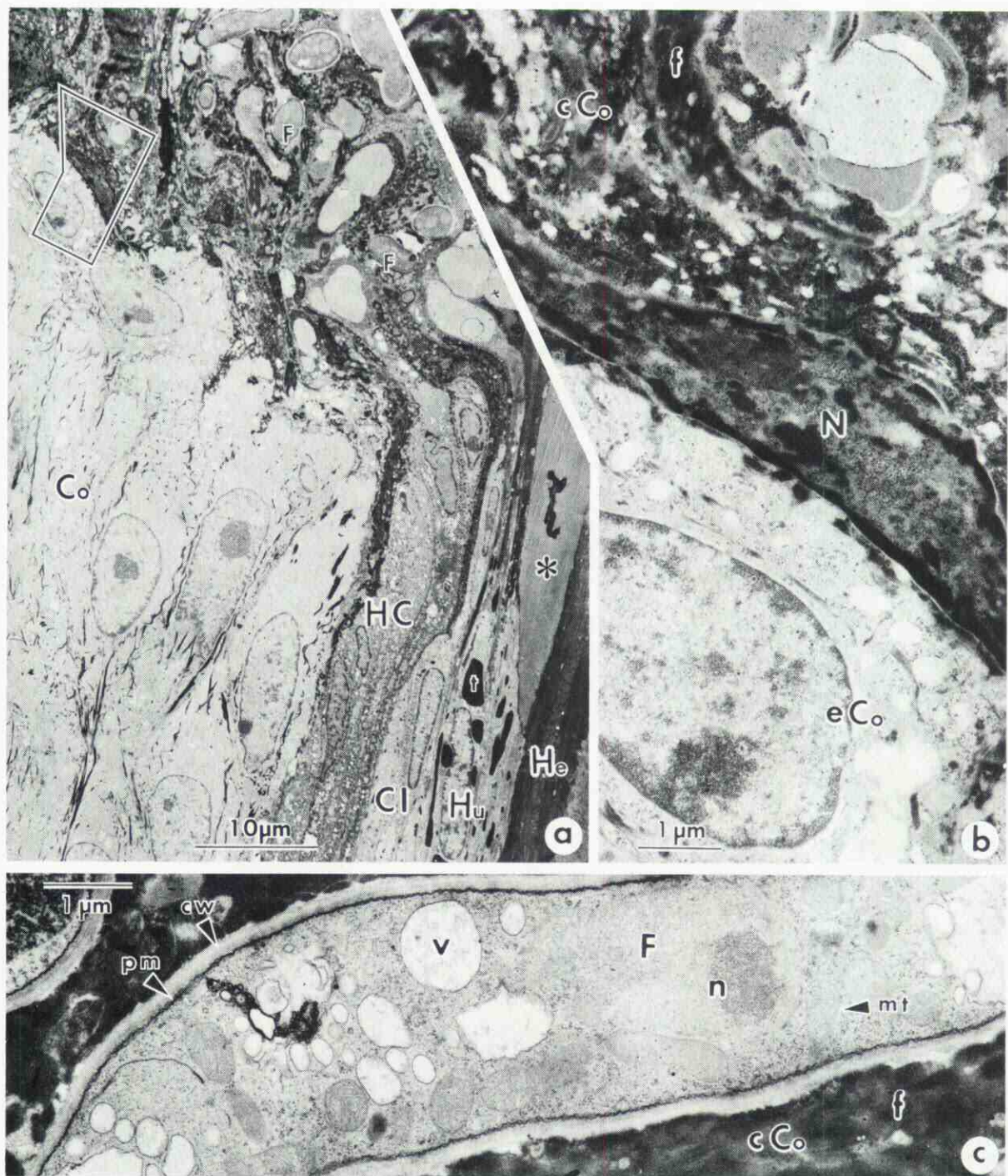
## DISCUSSION

Previous investigators reported from LM findings that in *tinea capitis* the downward growth of the intrapillary hyphae was abruptly terminated at the upper limit of keratogenous zone [1] and that fungi did not reach the keratogenous zone [2]. These concepts should be modified or corrected from the present TEM findings.

In the lower keratogenous zone of the cortex, the fungal elements were in the form of nonseptate hyphae with less electron density and stainability by toluidine blue. They were considered to exist continuously in the keratogenous zone against the upward stream of the differentiating and keratinizing cortical cells; this form may be a frontier form of fungi invading the keratinizing tissues and a proliferative form of fungi producing septate hyphae and spores. Furthermore, the fungi of this form may excrete some enzymes [20–22] or toxic substances into the surrounding tissues, because degenerative

septate hyphae usually contained moderately electron dense sub-round septal granules [15–18] of 0.15–0.2  $\mu\text{m}$  diameter in the cytoplasm near the septal pores (Fig 4*c*). Besides, several round, electron dense bodies were irregularly scattered in the cytoplasm; these dense bodies were composed of fine granular or homogeneous substances with varied electron densities, occasionally had a limiting membrane, and measured from 0.1  $\times$  0.1 up to 5.8  $\times$  2.6  $\mu\text{m}$  in size (Fig 4*a–c*). The septate hyphae infrequently showed a partial degeneration (Fig 4*a*). At the isthmus level where the outer root sheath cells were keratinizing, the fungal elements were transformed into round to elliptical arthrospores of 3–6  $\mu\text{m}$  diameter. They showed an increased electron density and had no dense body in the cytoplasm. The cell wall of the arthrospores was about 0.3–1.0  $\mu\text{m}$  in thickness, and was composed of an electron dense multilayered fibrillar inner structure and a less electron dense more amorphous unequal outer layer. Around the fungal elements, halolike, fiber-, and melanosome-free spaces with or without amorphous materials were observed; in the spaces broken or thinned, keratin bundles and a few melanosomes scattered (Fig 5). When the cross-sections of the affected hairs at the same level were observed, the hair shafts showed a slightly, but significantly, decreased diameter and an undulation of the hair cuticle layers.





**Figure 6.** TEM findings of the deeply affected hair tissue. *a*, longitudinal section of the hair tissue at the level just above hair bulb. The hair cortical cells (*Co*) show cell edema and, then, condensation and pyknotic change near the fungal elements (*F*). *b*, enlargement of the area indicated by the enclosure in *a*. *c*, nonseptate fungal element (*F*) in the condensed cortical cell (*cCo*). Uranyl acetate-lead citrate stain. Asterisk, keratinized Huxley's layer; *CI*, cuticle of inner root sheath; *cw*, cell wall; *eCo*, edematous hair cortical cell; *f*, aggregated tonofilaments; *HC*, hair cuticle; *He*, Henle's layer; *Hu*, Huxley's layer; *mt*, mitochondrion; *N*, pyknotic nucleus of the condensed hair cortical cell; *n*, nucleus of fungal element; *pm*, plasma membrane; *t*, trichohyaline granule; *v*, vacuole. *a*,  $\times 1,900$ ; *b*,  $\times 13,200$ ; *c*,  $\times 14,000$

changes of the cortical cells such as vacuolation, aggregation of tonofilaments, nuclear condensation, and cell edema were seen (see results). The fungi may produce some enzymes digesting melanin substances as well as enzymes digesting keratin substances. However, the fungi do not seem to influence or prevent the generation of the cortical cells. There may be a balance between the fungus proliferation and the cortical cell development [1].

With the upward movement of hair cortical cells, the fungi changed their structures, finally to arthrospores in the hair cortex. During this transformation of fungi, the keratinized cortical tissue became more digested and fiber- and melanin-free spaces gradually extended, resulting in a decrease of volume of the hair shaft. With this destruction of the hair shaft by the fungi, some of arthrospores may come out into the hair canal; when the hair shaft is more



severely destroyed and more spores are produced, the hair shaft structure has been lost and the hair canal is filled with the spores. The changes of hair clinically observed are considered to depend upon the number of proliferated fungal elements and the degree of tension or strength loss in the affected hairs.

The morphological characteristics of the septate hyphae in the hair tissue are of interest; ultrastructurally, many dense bodies were usually observed in the septate hyphae. The dense bodies seem to be different either from the septal granules [15–18] or peripheral bodies [23,24] near the septal pores or from the cortical melanosomes. They seem to be morphologically distinguished from the dense inclusions of glycogen or lipid in the fungal cytoplasm [25]. Morphologically, they resemble secondary lysosomes and may be derived from the digested and absorbed substances of the hair cortical cells. To resolve what the dense bodies are, further studies are required.

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